ANALYTICAL PART REPORT

SILRES® BS 1701

Analytical Part to:
Prenatal Developmental Toxicity Study in the Han Wistar Rat

Subtitle:
Determination of Content and Homogeneity in Application Formulations

Study Scientist: Dr. D. Flade

Test Facility: **Harlan Laboratories Ltd.**
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84489 Burghausen / Germany

Study Identification: Harlan Laboratories Study **C16992**

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PREFACE

General Information

Test Item:	SILRES® BS 1701
Analytical Part to:	Prenatal Developmental Toxicity Study in the Han Wistar Rat
Sponsor:	Wacker Chemie AG Johannes-Hess-Strasse 24 84489 Burghausen / Germany
Test Facility:	Harlan Laboratories Ltd. Wölferstrasse 4 4414 Füllinsdorf/Switzerland

Responsibilities

Study Scientist Analytical Chemistry:	Dr. D. Flade
Reporting:	R. Martone
Head of QA:	T. Fink

Schedule of Analytical Part

Experimental Starting Date:	12-Mar-2009
Experimental Completion Date:	31-Mar-2009

1 SUMMARY

This analytical part was conducted at Harlan Laboratories Ltd., Itingen, Switzerland under GLP-compliant conditions to verify the identity of the test item SILRES® BS 1701 administered and to determine the content and homogeneity of application formulations.

Several application formulations were prepared and representative analytical samples were collected and dispatched to the analytical laboratories internally. The test item concentrations were determined by gas chromatography coupled to a flame ionization detector and quantified with the area under the peak.

The identity of SILRES® BS 1701 was confirmed by its retention time which was similar to that measured in the working standards. The test item content in all samples was found to be within the accepted range of ±20% of the nominal content. In addition, the homogeneous distribution of SILRES® BS 1701 in corn oil was demonstrated.

In conclusion, the results obtained within this part confirm the correct preparation of application formulations during the conduct of this study.

2 PURPOSE

Within this analytical part the accurate preparation of application formulations during the study should be verified. For this purpose representative samples were analyzed for identity of SILRES® BS 1701, content and homogeneous distribution of the test item application formulations.

3 MATERIALS AND METHODS

3.1 Test Item

Detailed information concerning the test item is provided in the main study report. It was also used as analytical standard.

3.2 Sampling and Storage

Application formulations were prepared for each dose group. Representative samples were derived by weighing approximately 2 g into glass vials. On the first treatment day samples for content and homogeneity determination were collected on each occasion from the top, middle and bottom of the mixing beakers. During the last week of the treatment samples were taken from the middle to confirm concentration. The samples were dispatched to the analytical laboratories internally and stored frozen at -20 ± 5 °C until analysis.

3.3 Reagents and Materials

Acetone: Baker no. 9254

3.4 Analytical Procedure

3.4.1 Preparation of Standard Solutions

Stock solutions of SILRES® BS 1701 in acetone were prepared for external standard calibration. For example, 21.09 mg of SILRES® BS 1701 was exactly weighed into a 50 mL volumetric flask and approximately 40 mL of dilution solvent was added. Then, the mixture was sonicated for 5 minutes and the flask was brought to volume with dilution solvent to yield a solution with a concentration of 421.8 µg/mL. Aliquots of this stock standard solution were used to prepare six working standard solutions in dilution solvent with a concentration range of 10.55 to 105.5 µg/mL. On each occasion at least twelve standard solutions derived from two stock standard solutions were used for calibration.

3.4.2 Analysis of Samples

The samples received were dissolved in acetone by sonication for 5 minutes and then diluted to volume with dilution solvent. The sample solutions were further diluted with dilution solvent into the calibration range.

3.4.3 Gas Chromatographic Conditions

GC:	AGILENT 6890	
Sampling Unit:	AGILENT 7683	
Column:	VF WAX MS (Varian), 30 m x 0.25 mm x 0.25 µm	
Carrier Gas:	Helium, 1.5 mL/min, constant flow	
Injection:	1 µL, splitless	
Detector:	FID	
Temperatures:	Injector:	300 °C
	Detector:	325 °C
	Oven:	50 °C for 1 min
	at	25 °C/min to 260 °C
		260 °C for 0 min

3.4.4 Evaluation of Results

Injected samples were quantified by comparing peak areas of SILRES® BS 1701 with reference to the calibration curve. The latter was obtained by correlation of the peak areas of the working standards with their corresponding concentrations (µg/mL), using the linear regression model following equation 1:

$$y = a + b \cdot x \qquad (1)$$

where

y	=	Response of SILRES® BS 1701
a	=	Intercept derived from linear regression of calibration data
b	=	Slope derived from linear regression of calibration data
x	=	Actual concentration of SILRES® BS 1701 in sample aliquot [µg/mL]

Sample aliquot concentrations were corrected for density of the application formulation and for dilution using equation 2:

$$c_{Actual} = \frac{x \cdot V \cdot D}{W \cdot 1000} \qquad (2)$$

where

c_{Actual}	=	Actual sample concentration [mg/mL]
x	=	Actual concentration of SILRES® BS 1701 in sample aliquot according to equation 1 [µg/mL]

V = Dilution volume [mL]
D = Density of application formulation [0.92 g/mL; density of vehicle]
W = Sample weight [g]

The sample recovery was determined as follows:

$$R = \frac{c_{Actual}}{c_{Nominal}} \cdot 100 \qquad (3)$$

where
R = Sample recovery [%]
c_{Actual} = Actual sample concentration [mg/mL]
$c_{Nominal}$ = Nominal sample concentration [mg/mL]

4 RESULTS

The linearity of the analytical systems used for sample analyses was demonstrated with a good relationship between peak areas measured and working standard concentrations. All calibration points used met the acceptance limit of ±20% variation from the calibration curve derived by linear regression analysis. The regression coefficients calculated were found to be better than 0.99. An example is presented in Figure 1.

The SILRES® BS 1701 peak was assigned in sample chromatograms by comparison to that of working standards. In blank sample chromatograms no peak appeared at the retention time of SILRES® BS 1701 and, therefore, it was confirmed that only corn oil was administered in the control part of the experiment. Examples of chromatograms are shown in Figure 2 and Figure 3.

The application formulations investigated during the study were found to comprise SILRES® BS 1701 in the range of 96.7% to 102.2% and, thus, the required content limit of ±20% with reference to the nominal concentration was met. The homogeneous distribution of SILRES® BS 1701 in the preparations was approved because single results found did not deviate more than 1.9% (<15%) from the corresponding mean.

In conclusion, the results indicate the accurate use of the test item SILRES® BS 1701 and corn oil as vehicle during this study. Application formulations were found to be homogeneously prepared. Detailed results are shown in Table 1.

Table 1 Detailed Results of Application Formulation Analysis
(Rounded results presented are based on calculations with exact data)

Dose Group	Sample taken from/after	Date of Analysis	Nominal Concentration [mg/mL]	Actual Concentration [mg/mL]	Recovery	Mean Recovery	Maximum Variation from Mean
Date of Preparation: 09-Mar-2009							
1	vehicle	16-Mar-09	0	0.000	---	---	---
2	top	16-Mar-09	20.0	19.55	97.8%	99.5%	1.8%
	middle	16-Mar-09	20.0	20.18	100.9%		
	bottom	16-Mar-09	20.0	19.98	99.9%		
3	top	16-Mar-09	60.0	58.85	98.1%	99.5%	1.4%
	middle	16-Mar-09	60.0	60.50	100.8%		
	bottom	16-Mar-09	60.0	59.66	99.4%		
4	top	16-Mar-09	200.0	199.3	99.7%	101.6%	1.9%
	middle	16-Mar-09	200.0	204.3	102.2%		
	bottom	16-Mar-09	200.0	205.7	102.8%		
Date of Preparation: 23-Mar-2009							
1	vehicle	31-Mar-09	0	0.000	---	---	---
2	middle	31-Mar-09	20.0	19.34	96.7%	---	---
3	middle	31-Mar-09	60.0	60.00	100.0%	---	---
4	middle	31-Mar-09	200.0	204.0	102.0%	---	---

Figure 1 Example of Calibration Curve

Date of analysis: 16-Mar-2009

Standard Concentration [µg/mL]	Peak Area [counts]	Variation of Peak Area
10.55	151.46	-2.8%
21.09	300.91	0.1%
42.18	582.69	-1.3%
63.27	879.11	-0.2%
84.36	1'168	-0.2%
105.5	1'453	-0.6%
9.957	141.43	-4.3%
16.60	234.21	-2.0%
33.19	466.62	-0.1%
66.38	921.14	-0.2%
82.98	1'151	0.0%
99.57	1'374	-0.4%
10.47	155.27	0.5%
41.86	596.65	1.8%
62.79	906.32	3.6%

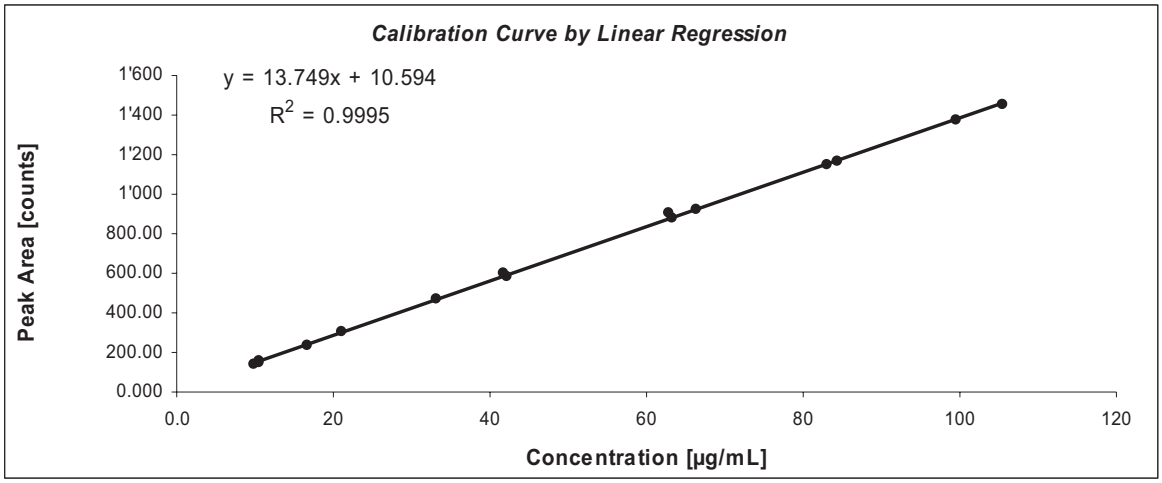
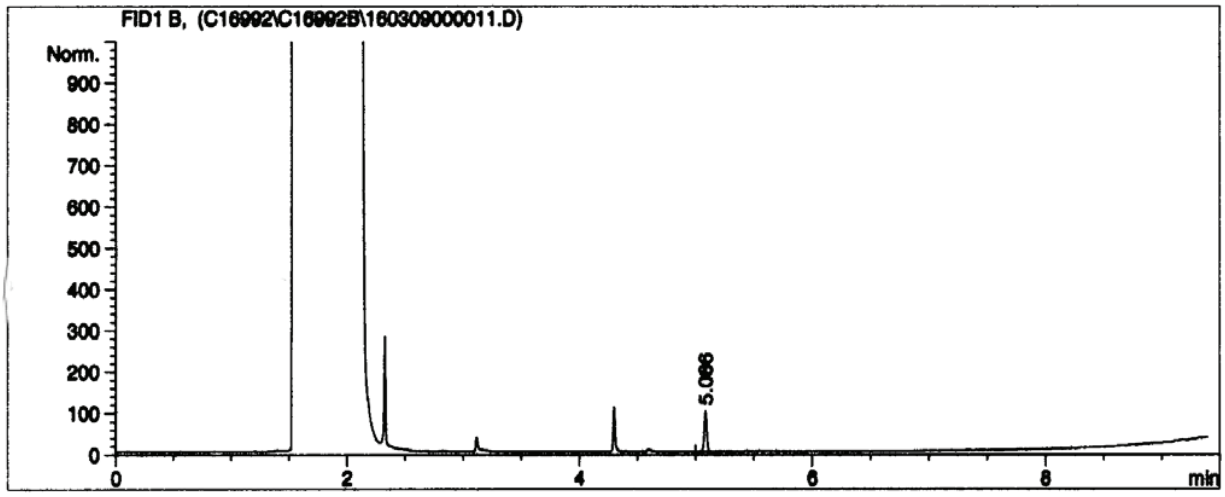


Figure 2 Typical GC-Chromatograms of Standard Solutions

(A) Standard solution: 10.55 µg/mL
(B) Standard solution: 105.5 µg/mL
Date of analysis: 16-Mar-2009

(A)



(B)

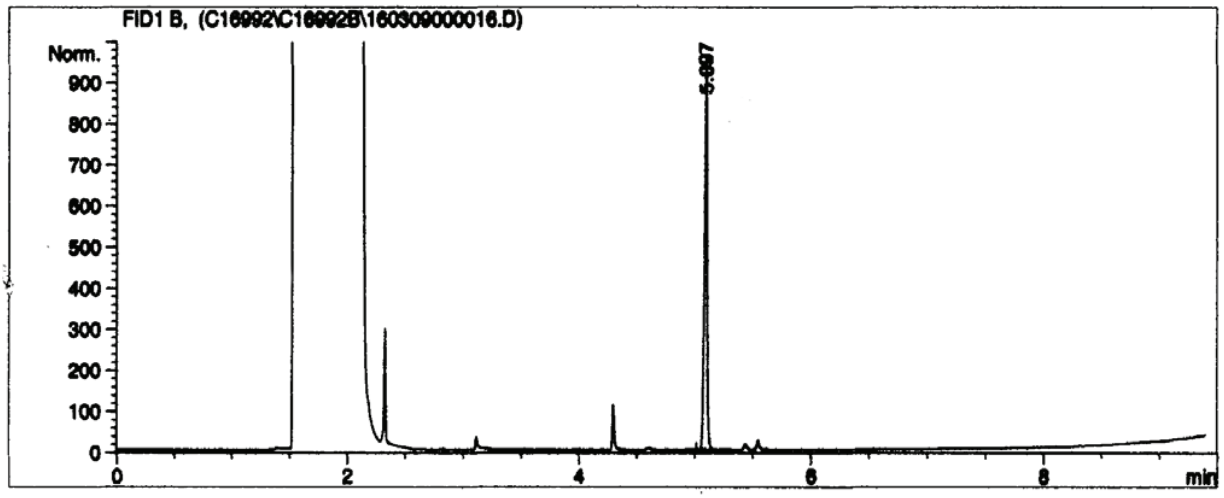
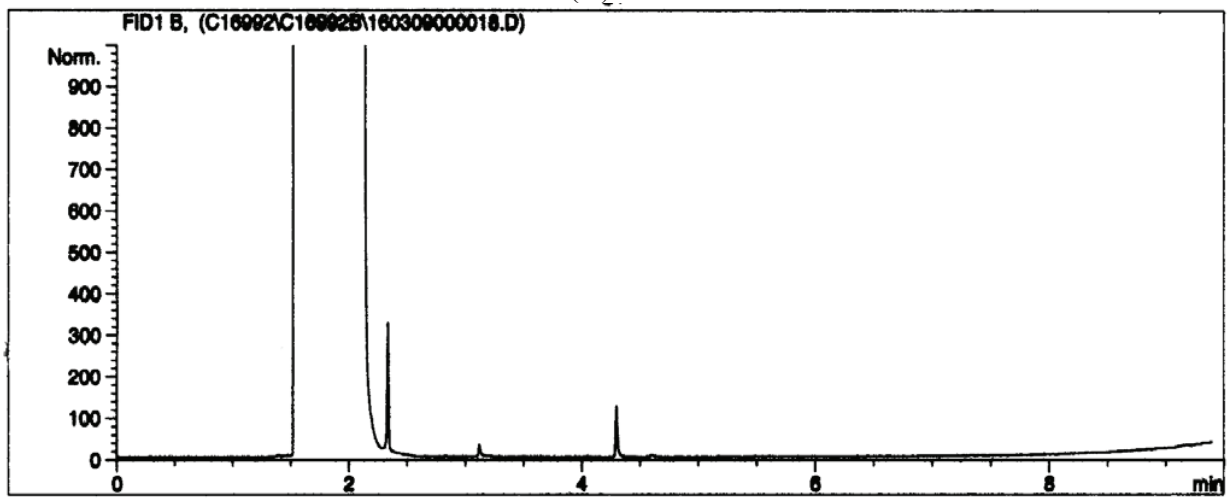


Figure 3 Typical GC-Chromatograms of Test Samples

(A) Dose group 1, control sample, 496x diluted
(B) Dose group 2 (top), nominal content: 20.0 mg/mL, 487x diluted
Date of analysis: 16-Mar-2009

(A)



(B)

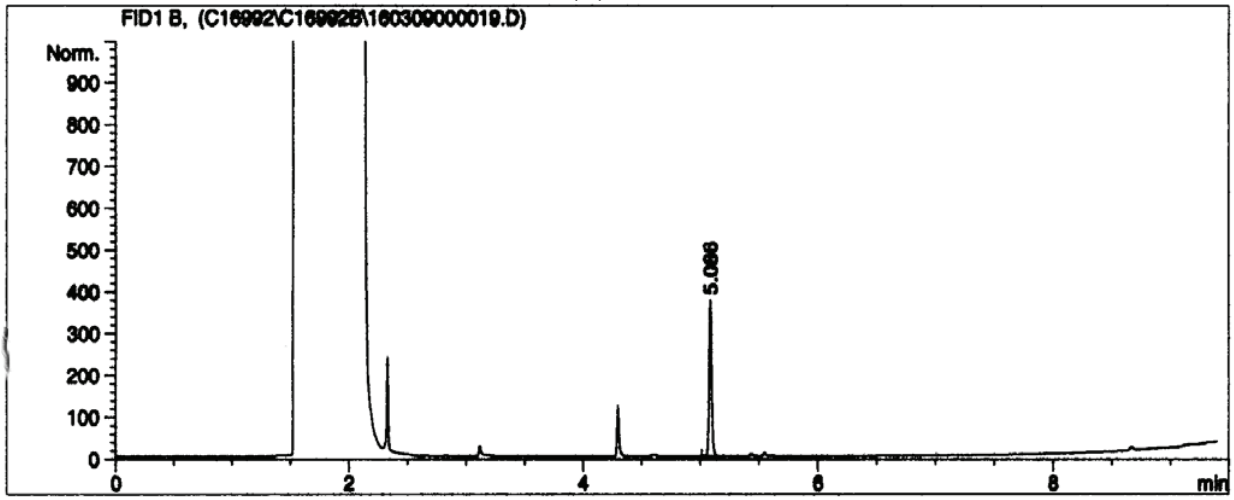


Figure 3 Cont'd

(C) Dose group 4 (top), nominal content: 200.0 mg/mL, 4824x diluted
Date of analysis: 16-Mar-2009

(C)